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(71) Applicant: **Kishimoto, Tadamitsu**
5-31 Nakanocho 3-chome
Tondabayashi-shi Osaka(JP)

(72) Inventor: **Kishimoto, Tadamitsu**
5-31 Nakanocho 3-chome
Tondabayashi-shi Osaka(JP)

(74) Representative: **Kearney, Kevin David Nicholas et al**
KILBURN & STRODE 30 John Streeteet
London, WC1N 2DD(GB)

(54) Human GP130 protein.

(57) Human gp130 protein having at least the following properties: (1) the protein has an affinity with a complex of IL-6 (interleukin-6) and an IL-6 receptor (interleukin-6 receptor); (2) the protein shows an apparent molecular weight of 130 kDa in SDS-polyacrylamide electrophoresis; and (3) the protein participates in the transmission of IL-6 signal; DNA coding for the protein, an expression plasmid containing the DNA; and a process for production of the protein using the expression plasmid.

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HUMAN GP130 PROTEIN

The present invention relates to a human gp130 protein, which is a protein participating in the transmission of an IL-6 signal, a DNA coding for this protein, and a means for and a method of producing this protein by genetic engineering.

Interleukin-6 (IL-6) is a protein participating broadly in the proliferation and differentiation of an organism, and is characterized in that the protein plays an important role in the defense system of an organism such as immunity, hematopoiesis or inflammation (see Kishimoto, Blood, 74, page 1, 1989). It is reported that an abnormal production of IL-6 may become a factor in the cause of various autoimmune diseases (see Kishimoto and Hirano, Ann. Rev. Immunol., 6 page 485, 1988).

The inventor isolated a gene for human IL-6 receptor on the cell membrane, which is specifically bonded to human IL-6, and determined the primary structure thereof (see Japanese Patent Application No. 63-194885). Then the inventor isolated a gene of mouse IL-6 receptor on the cell membrane, which is specifically bonded to mouse IL-6, and determined the primary structure thereof (see Japanese Patent Application No. 1-292230). Furthermore, the inventor prepared a soluble IL-6 receptor (extracellular portion of an IL-6 receptor), considered to be usable for a therapeutic or diagnostic medicine, from the human IL-6 receptor gene as the starting material (see Japanese Patent Application No. 1-9774).

An artificial control of the function of IL-6 strongly exerting physiological activities in an organism is considered usable as a new mechanism for the therapy of various diseases, and knowledge of the course of the transmission of an IL-6 signal is important in the development of a medicinal substance for enhancing or inhibiting the function of IL-6.

The inventor investigated the mechanism of the transmission of the IL-6 signal, and as a result, found that, in addition to the IL-6 and IL-6 receptor, there exists, as a third factor, a protein participating in the transmission of the IL-6 signal. Since this protein has an apparent molecular weight of 130 kb, the inventors named his protein "human gp130". To further analyze the transmission of IL-6 signals or to develop a soluble gp130 (the extracellular portion of a gp130 protein) as an inhibitor for IL-6, a large quantity of a purified product of soluble gp130 must be obtained but the amount of gp130 produced in an organism is very small. A DNA sequence coding for gp130 is indispensable for producing gp130 in a large quantity by a genetic engineering method.

Therefore, a primary object of the present invention is to provide a human gp130, a DNA coding for the gp130, and a process for producing the gp130 by using the DNA.

The inventor carried out research into the transmission of the IL-6 signal and found a protein on the cell membrane which participates in the transmission of IL-6 signals, and furthermore, proved that the IL-6 receptor on the cell membrane is bonded to this membrane protein through the bonding to IL-6, this membrane protein mediates the transmission of the IL-6 signal, and the intracellular region of the IL-6 receptor does not participate in the transmission of IL-6 signals.

Also, the inventor succeeded in cloning a DNA coding for human gp130, and determined the nucleotide sequence of this DNA.

The present invention is based on the results of these researches. More specifically, the present invention provides a gp130 participating in the transmission of the IL-6 signal; a DNA sequence coding for human gp130; a replicable vector capable of expressing this DNA sequence in a recombinant microorganism or cultured cell; a microorganism or cultured cell transformed by this expression vector; and a process for the production of human gp130, which comprises expressing a DNA sequence coding for gp130 in this microorganism or cultured cell.

Figure 1 shows autoradiographic patterns obtained when U266 cells internally labelled with ^{35}S-methionine are lysed after treatment with IL-6 (lanes 2 and 4) or without treatment with IL-6 (lanes 1 and 3), and then immunoprecipitated by an IL-6 receptor antibody MT18 immobilized on Sepharose B4, and polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS/PAGE) is carried out under non-reducing conditions (lanes 1 and 2) or under reducing conditions (lanes 3 and 4);

Figure 2 shows autoradiographic patterns obtained when M12IL6R cells surface-labelled with ^{125}I are lysed after treatment with IL-6 (lane 2) or without treatment with IL-6 (lane 1), and is then treated in the same manner as in Figure 1;

Figure 3 shows autoradiographic patterns obtained when M12 cells are incubated in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of IL-6 and in the presence of a culture supernatant of COS7 cells containing solluble IL-6 receptor (lanes 3 and 4) or not containing soluble IL-6 receptor (lanes 1 and 2) and lysed, and is then treated in the same manner as in Figure 1;

Figure 4 shows incorporation of ^{3}H-labelled thymidine by MI cells in the presence of IL-6 at various concentrations, and in the presence or absence of a soluble IL-6 receptor;

Figure 5 shows a restriction enzyme map of λA and λB and a schematic structure of a corresponding human gp130 protein, in which E represents the EcoRI site, SS represents the signal sequence, EC represents the extracellular region, TM represents the membrane penetration region, and C represents the intracellular region;

Figure 6 shows the structure of a plasmid pGP130;

Figures 7-1 through 7-6 show the results of the analysis of the nucleotide sequence (Sequence ID N°. 1) of insert DNA of pGP130, i.e. DNA coding for human gp130, and show the presumed amino acid sequence of a protein, i.e. human gp130 produced when this DNA sequence is expressed, in which the underlined portion represents a hydrophobic amino acid region on the N-terminal side and the double-underlined portion represents a hydrophobic amino acid region on the C-terminal side; and,

Figure 8 shows the results of a Northern blot analysis of various cells conducted by using human gp130 CDNA as the probe.

1. gp130 Protein.

The cell membrane-derived protein participating in the transmission of the IL-6 signal according to the present invention, i.e. gp130, exerts an important function when IL-6 is bonded to an IL-6 receptor to finally cause various cells to exert biological activities such as proliferation and differentiation, and in the natural state, this protein is located on the cell membrane. The IL-6 referred to herein includes IL-6 produced in an organism, IL-6 produced by a genetic engineering method, and derivatives thereof. Furthermore, the IL-6 receptor includes an IL-6 receptor present on the cell membrane produced in an organism or by a genetic engineering method, a soluble IL-6 receptor isolated from the cell membrane, and derivatives thereof.

This protein has the following properties.

(1) The protein is bonded to a complex of IL-6 and an IL-6 receptor.

(2) The protein is not bonded to IL-6 alone or IL-6 receptor alone.

(3) The protein shows an apparent molecular weight of 130 kDa in the SDS/PAGE. This protein participates in the transmission of the IL-6 signal and in the natural state, has an amino acid sequence (referred to herein as formula (I) of from Met at the 1-site to Gln at the 918-site as shown in Figure 7. In the present invention, the protein may be any protein having the above-mentioned amino acid sequence, and a protein or polypeptide having a portion of the above-mentioned amino acid sequence that makes a contribution to the specific bonding to a complex of IL-6 and IL-6 receptor. Namely, all proteins and polypeptides having an amino acid sequence in which at least one amino acid residue of the above-mentioned amino acid sequence is substituted by another amino acid residue(s), or at least one amino acid is deleted from the above- mentioned amino acid sequence, or at least one amino acid is added to the above-mentioned amino acid sequence, whereby the protein still retains a capacity of being specifically bonded to a complex of IL-6 and IL-6 receptor, are included within the scope of the pg130 of the present invention. For example included within the term "pg130" are proteins in which an amino acid sequence or amino acid residue not making a contribution to the bonding to the complex of IL-6 and IL-6 receptor in the above-mentioned amino acid sequence is modified by substitution, deficiency or insertion; proteins in which an amino acid sequence and/or an amino acid residue portion is added to the N-terminal side and/or the C-terminal side of the above-mentioned amino acid sequence; and proteins formed by a fusion of another protein, such as a human growth hormone, to the above-mentioned amino acid sequence.

The amino acid sequence represented by formula (I) consists of 918 amino acid residues, and hydrophobic amino residues are located in the region of from leucine at the second position from the N-terminal side to glycine located at the 22nd position and the region of from alanine at the 620th position to phenylalanine at the 641st position. It is considered that, of these two regions, the former region is a signal peptide region and the latter region is a membrane penetration region.

2. DNA Sequence.

DNA coding for human gp130 protein of the present invention encodes, for example, an amino acid sequence of from Met at 1-position to Gln at 918-position as shown in Figure 7. Representative cDNA has a base sequence of from A at 273-position to G at 3026- position as shown in Figure 7. The present invention covers not only the above-mentioned DNA sequence but also DNA in which at least one nucleotide of the above-mentioned DNA sequence is substituted with another nucleotide, or at least one nucleotide is deleted from the above-mentioned DNA sequence, or at least one nucleotide is added to the above-mentioned DNA sequence, and which codes for a protein having a capacity of being specifically bonded to the IL-6/IL-6 receptor complex. For example, there can be mentioned DNA sequences coding for proteins having the amino acid sequences as men-

tioned in formula (I) above.

3. Process for Preparation of gp130 Protein.

According to a first process of the present invention, the cell membrane-derived protein participating in the transmission of the IL-6 signal is prepared from animal cells producing this protein. As the cells, there can be mentioned IL-6 receptor-producing cells, for example, human myeloma cell U266, mouse leukemia cell line M1, and human B cell line CL4. The protein of the present invention participating in the transmission of the IL-6 signal also can be produced by the cells not producing IL-6 receptor. As the cells of this type, mouse B cell line M12, human T cell line Jurkat, and mouse T cell line CTLL2 can be mentioned. Namely, the protein participating in the transmission of the IL-6 signal according to the present invention can be obtained from these cells.

As pointed out hereinbefore, the protein of the present invention is bonded to the IL-6 receptor in the presence of IL-6 but is not bonded to the IL-6 receptor in the absence of IL-6, and therefore, the protein of the present invention can be isolated by utilizing this property.

For example, to isolate the protein from cells producing both the IL-6 receptor and the protein of the present invention, a process can be adopted in which the producing cells are cultured, IL-6 is artificially added to the cultured cells, incubation is carried out for a time sufficient to allow a reaction between the cells and the added IL-6 under physiological conditions, for example, for about 30 minutes at about 37° C in a conventional culture medium such as RPMI1640, and the cells are then lysed by a customary method, for example, by a treatment with a triethanolamine buffer solution containing 1% of digitonine, whereby a cell lysate containing a complex in which IL-6, the IL-6 receptor and the protein of the present invention are bonded together is obtained. Alternatively, after the cells are lysed by a customary method, incubation is carried out in the above-mentioned manner to obtain a cell lysate containing the above-mentioned complex. An antibody to IL-6 receptor, for example, MT18 antibody (prepared according to Referential Example 1 given hereinafter; see Japanese Patent Application No. 63-194885) is immobilized by a solid carrier, for example, Sepharose 4B, by the cyanogen bromide activation method. By placing this immobilized antibody to IL-6 receptor in contact with the above-mentioned cell lysate, the complex containing the protein of the present invention is immobilized on the above-mentioned solid carrier through the specific reaction between the IL-6 receptor and the IL-6 receptor antibody. Then, this solid carrier is washed to remove non-specifically bonded or adhering impurities, and the complex is eluted from the solid carrier by a conventional means using, for example, urea or guanidine, to free the desired protein of the present invention from the IL-6 receptor-containing protein complex.

To isolate and purify the desired protein of the present invention from the obtained solution, customary means adopted for the isolation and purification of proteins, such as precipitation using ammonium sulfate, column chromatography, for example, reversed phase chromatography and ion exchange chromatography, and electrophoresis can be used. During this isolation and purification process, an active fraction can be selected by utilizing the bonding to the IL-6 receptor in the presence of IL-6, non-bonding to the IL-6 receptor in the absence of IL-6, and confirming the molecular weight of 130 kDa as the criterion.

Where cells producing the protein of the present invention but not producing the IL-6 receptor are used, incubation is carried out in the presence of a soluble IL-6 receptor and IL-6, and the cells are then lysed to obtain a cell lysate containing an IL-6/IL-6 receptor/desired protein complex. Alternatively, the abovementioned cells producing the protein of the present invention are lysed, an incubation of IL-6 and the IL-6 receptor is carried out, and finally, a cell lysate containing an IL-6/IL-6 receptor/desired protein complex is obtained. The desired protein can be isolated and purified from this cell lysate in the above-mentioned manner.

The second process for the production of the protein participating in the transmission of IL-6 signals according to the present invention utilizes gene recombination, or recombinant DNA techniques. In this process, DNA coding for an amino acid sequence of a protein on the cell membrane, which participates in the transmission of the IL-6 signal can be obtained by various methods. For example, a cDNA library may be formed from cells expressing the above-mentioned protein thereon, such as human myeloma cells, according to customary procedures, and this library is selected according to various methods, for example, the method in which a probe designed on the basis of a partial amino acid sequence of the purified protein is used, and the method in which cDNA is expressed and the selection is made based on the property of the produced protein, for example, the specific bonding to the IL-6-bonded IL-6 receptor. The protein of the present invention is produced by expressing the thus-cloned DNA according to customary procedures.

4. DNA Coding gp130 Protein.

The DNA to be used for the above-mentioned process can be prepared based on desired messenger RNA selected from various messenger RNA's extracted, for example, for the human placenta by a known method, by using a probe prepared based on the DNA sequence of Figure 7 provided by the present invention. Alternatively, a part or all of this DNA can be chemically synthesized according to the disclosure of the present invention.

5. Expression Vector.

As the replicable expression vector capable of expressing the DNA sequence coding human gp130 provided according to the present invention, any vector having a replicating origin, control sequences and a sequence coding for gp130, and capable of transforming a selected host can be appropriately used without limitation. For example, plasmids such as pBR322 and pBR327 can be used when E. coli is used as the host, and when cultured cells of a mammal or the like is used as the host, a vector having a DNA replicating origin derived from SV40 virus, can be used.

Of the control sequences for expressing the coding sequence, the promoter system is important, and an appropriate promoter system should be elected according to the relationship to the selected host. When E. coli is used as the host, a lactose promoter system, a tryptophan promoter system or hybrid promoter systems thereof are preferably selected. When cultured cells derived from a mammal are used as the host, an SV40 promoter system, adenovirus promoter system, and cytomegalovirus promoter system are preferably selected.

6. Host.

Microorganisms or cultured cells customarily used for producing proteins by genetic engineering can be used as the host without limitation. As the microorganism, there can be mentioned E. coli such as K-12 or W-3110, Bacillus subtilis and yeast, and as the cultured cells, there can be mentioned COS cells (monkey renal fibroblast), CHO cells (Chinese hamster oocyte), and myeloma cells.

The host explained in (6) above, which has been transformed by the vector explained in (5) above, is cultured to express the DNA sequence coding for human gp130 in the vector DNA, whereby human gp130 is produced. This is realized by the activation of the promoter system in the control sequences linked to the DNA sequence coding for human gp130.

A polyclonal antibody or monoclonal antibody to the protein of the present invention can be prepared according to customary procedures by using the protein of the present invention prepared according to various processes as described above, or the cell producing this protein as an immunogen. As the cell source and animal to be used in this preparation process, there can be mentioned organisms such as human, mouse, rabbit, goat and sheep.

The cell membrane-derived protein participating in the transmission of the IL-6 signal provided according to the present invention, can be used as a reagent for clarifying the signal transmission mechanism of IL-6 and developing valuable substances such as therapeutic agents for controlling the function of IL-6. For example, by using, as a detection criterion, the property of the present protein which transmits IL-6 signal while being specifically bonded to the IL-6 receptor bonded to IL-6, substances controlling the function of IL-6 can be screened. As the substances to be screened, there can be mentioned natural substances, synthetic chemicals, IL-6 and an IL-6 receptor produced by genetic engineering, derivatives thereof, and antibodies to IL-6, the IL-6 receptor, and antibodies to these proteins.

Furthermore, by bonding the soluble IL-6 receptor which has been bonded to IL-6 to the present protein, the function of IL-6 can be enhanced, and the function of IL-6 can be exerted even on a cell having no IL-6 receptor in the cell surface layer. On the other hand, if the bonding of the above-mentioned protein to the IL-6 receptor which has been bonded to IL-6 is inhibited by an antibody to IL-6, IL-6 receptor or the above-mentioned protein, the biological activity of IL-6 will be inhibited. Accordingly, it is considered that the protein of the present invention will be usable as an active ingredient of a medicine.

Furthermore, by the DNA sequence coding for human gp130 according to the present invention, and the means and process for producing this protein by genetic engineering, this protein, which is produced only in a very small amount in the natural state, can be produced in a large quantity.

The present invention will now be described in detail with reference to the following examples, that by no means limit the scope of the invention. It should be noted that the protein of the present invention is sometimes called "gp130" in the examples for convenience.

Example 1

Bonding of Protein (gp130) on Human Cell Membrane to Human IL-6 Receptor in Presence of Human IL-6

Human myeloma cells U266 (cells producing the IL-6 receptor and gp130 protein) (2 x 10^7 cells) were internally labelled with 1 mCi of ^{35}S-methionine and divided into two parts. One part was incubated in 0.5 ml of PRMI1640 culture medium at 37° C for 30 minutes in the presence of IL-6 (1 ug/ml), and the other part was similarly incubated in the absence of IL-6. Then, the U266 cells were lysed with 1 ml of a 10 mM triethanolamine buffer solution (having a pH value of 7.4) containing 1% of digitonine (supplied by Wako Junyaku), 0.15 M NaCl, and 1 mM pAPMSF (a commercially available plasmid supplied by Wako Junyaku). Separately, the IL-6 receptor antibody MT18 (Referential Example 1) was bonded to Sepharose 4B activated by cyanogen bromide, and the bonded antibody then mixed with the supernatant of the above-mentioned cell lysate, whereby the solubilized IL-6 receptor was bonded to the MT18 antibody on the resin.

The non-specifically bonded substances were washed away by the above-mentioned solution containing 1% of digitonine. Then, under reducing or non-reducing conditions, polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS/PAGE) and autoradiography were carried out. The results are shown in Figure 1. In Figure 1, the band of 80 kDa shows the ^{35}S-methionine-labelled IL-6 receptor derived from the U266 cell, and the band of 130 kDa shows the gp130 protein of the present invention. From the results shown in Figure 1, it is seen that the IL-6 receptor bonded to IL-6 is bonded to the human protein (gp130) having a monomer molecular weight of 130 kDa within 30 minutes at 37° C.

Example 2

Bonding of Human IL-6 receptor to Protein (gp130) on Mouse Cell Membrne in Presence of Human IL-6

A plasmid prepared by inserting a human IL-6 receptor cDNA into the BamHI site in the plasmid pZipNeoSV(X)I (see Cepko et al, Cell, 37, page 1053, 1984) was introduced into a mouse B cell strain M12 not expressing the IL-6 receptor on the cell surface, by the electropolation method, and the transformed cell was screened based on the resistance to an antibiotic substance G418 (neomycin analogue - supplied by Sigma). The thus-established mouse B cell M12-derived clone expressing the human IL-6 receptor on the cell surface was named "M12IL6R".

Then, 10^7 of M121L6R cells were washed with PBS and suspnded in 0.1 ml of 50 mM Tris (having a pH value of 7.4) and 0.15 M NaCl. Next, 1 mCi of Na ^{125}I and two Iodobeads (supplied by Pierce) were reacted at room temperature for 5 minutes in 0.1 ml of the above-mentioned buffer solution, the reaction liquid was mixed with the above-mentioned cell suspension, and incubation was carried out at room temperature for 30 minutes. Then 1 ug/ml of IL-6 was added to the thus-labelled cells, or was not added, and the reaction was carried out at 37° C for 30 minutes, and the cell was lysed according to the method described in Example 1, an immunoprecipitation by MT18 (see reference Example 1) was carried out, and SDS/PAGE and autoradiography were then carried out. The results are shown in Figure 2. In Figure 2, the band of 80 kDa shows the IL-6 receptor produced by the IL-6 receptor cDNA introduced into an M12 cell, and the band of 130 kDa shows a gp130 protein inherently produced by the M12 cell. From the results shown in Figure 2, it is seen that the human IL-6 receptor on M12IL6R bonded to human IL-6 is bonded to the mouse protein (gp130) derived from M12IL6R, at 37° C within 30 minutes.

Example 3

Bonding of Soluble IL-6 Receptor to gp130 in Presence of IL-6

According to the method described in Example 2, 2 x 10^7 of M12 cells (cells not producing IL-6 receptor) were labelled, 5 x 10^6 of the labelled cells were added to 1 ml of a culture supernatant of COS7 cells containing or not containing the soluble IL-6 receptor in the presence or presence of IL-6 (1 ug/ml), and the reaction was carried out at 37° C for 30 minutes. Then the cells were lysed with digitonine according to the method described in Example 1, immunoprecipitation was carried out by MT18 antibody, and the SDS/PAGE and autoradiography were then carried out. The results are shown in Figure 3. In Figure 3, the band of 130 kDa shows the gp130 protein produced by M12 cells. Note, in this example, since the soluble IL-6 receptor was radiography. From the results shown in Figure 3, it is seen that the complex of IL-6 and the soluble IL-6 receptor is bonded to the protein (gp130) on the cell membrane, and the soluble IL-6 receptor alone is not bonded to gp130.

Example 4

Enhancement of Function of IL-6 bv Soluble IL-6 Receptor in Presence of IL-6

M1 cells (1 x 10^5 cells/ml) were cultured at 0.2 ml/well in a culture medium containing Il-6 at various concentrations as well as COS7 cell supernatant or soluble IL-6 receptor-containing COS7 cell supernatant in an amount of 25%. Within 60 to 70 hours from the start of the culturing, ^{3}H-labelled thymidine was added, and by measuring the radioactivity incorporated into the cells, the proliferation of the cells was examined. Figure 4 shows that the effect of inhibiting the proliferation of M1 cells, possessed by IL-6, is enhanced by the soluble IL-6 receptor. Namely, the function of IL-6 is enhanced by the bonding of the IL-6-bonded soluble IL-6 receptor to gp130 of M1 cell.

Example 5

Isolation of Human gp130 cDNA

A series of gene recombintion operations (extraction of m-RNA, cleavage of DNA by restriction enzyme, and the like) were carried out according to the process of Maniatis et al (see Molecular Cloning, Cold Spring Harbor Laboratory, 1982) and the process of Harwin et al (see DNA Cloning, A Practical Approach, vol. 1, page 49, IRL, Oxford, 1985). Screening by the antibody of λgt11 cDNA library was carried out according to the conventional method.

First, a messenger (m) RNA was extracted from human myeloma cell U266 (see Cell, volume 58, page 573, 1989), and a cDNA library was prepared by using λ gt11 (supplied by Clontech). Then, using mouse-derived monoclonal antibodies AM64 and AM277 (see Japanese Patent Application No. 2-15090) to gp130, about 500,000 clones were screened, and finally, two colonies and (λ_A and λ_B) were obtained. The insert cDNA of each of λA and λB was analyzed, and the results are shown in Figure 5. As shown in Figure 5, the insert cDNAS did not contain the full length human gp130-coding region.

Accordingly, an EcoRI fragment of λ_A and the EcoRI fragment of λ_B were inserted, while connecting them in the orientation of reading of cDNA, to the EcoRI site of a vector pBluescript SK (supplied by Stratagene) to prepare a plasmid pGP130 having an insert DNA containing the complete human gp130-coding region. The structure of pGP130 is shown in Figure 6, and the sequence of the insert DNA of pGP130, i.e. the sequence of human gp130 cDNA determined from insert cDNA sequences of λA and λB, and the presumed amino acid sequence, are shown in Figure 7.

E. coli HB101/pGP130 containing this plasmid pGP130 was internationally deposited as deposition No. FERM BP-2912 under the Budapest Treaty on May 15, 1990 with the Fermentation Research Institute, Agency of Industrial Science and Technology, located at 1-3, Higashi 1-Chome, Tsujuka-shi, Ibaragi-ken, Japan.

Example 6

Expression of Human gp130 mRNA in Various Cells

To examine the expression of human gp130 in various cells, Northern blot analysis was carried out. The culturing of cells, extraction of mRNA from the cells, electrophoresis, blotting, probe labelling, hybridization, filter washing, and autoradiography were carried out according to standard methods.

mRNA was extracted from human myeloma cells U266, human B cells CESS, human Burkitt's lymphoma Jijoye, human T cells Jurkat, and NK cells YT, and 1 ug of each mRNA then denaturated by the formamide method and subjected to electrophoresis with 0.8% agarose gel, and to Northern blotting on a nylon filter membrane. Then an insert cDNA of λ_A obtained by the method described in Example 5 was extracted, and using this extracted insert cDNA, hybridization was carried out at 42° C for 24 hours. The product was then washed and autoradiography was carried out.

The results are shown in Figure 8, and it is seen that the manifestation of gp130 mRNA is strong in U266 and YT, medium in CESS, and weak in Jijoye and Jurkat. This has a relation to the reactivity of the IL-6 of each cell.

Referential Example 1

Preparation of Monoclonal Antibody to Human IL-6 Receptor

To prepare a mouse monoclonal antibody to human IL-6 receptor, mouse T cells having human IL-6 receptors expressed on the cell membrane surface were prepared as an antigen, according to the following method. Namely, pBSF2R.236 and pSV2neo disclosed in Japanese Patent Application No. 1-9744 were introduced in mouse T-cells CTLL-2 (ATCC, TIB214) according to a conventional procedure, and screening was carried out according to the conventional method using antibiotic G-418. Finally, the strain in which about

30,000 IL-6 receptors per cell were expressed was established, and this strain was named "CTBC2".

The immunization was carried out in the following manner. The CTBC2 cells were cultured according to a conventional method using PRMI1640 culture medium, and the cultured CTBC2 cells were washed four times with a PBS buffer and used intraperitoneally to immunize C57BL6 mouse in an amount of 1×10^7 cells per mouse once a week, six times as a whole.

The spleen cells of the immunized mouse were fused to the myeloma cells P3U1 as the parent cell line, according to a conventional method using polyethylene glycol.

Screening was carried out in the following manner. Namely, pBRF2R.236 and pSV2neo were introduced into the IL-6 receptor-negative human T cell strain JURKAT (ATCC, CRL8163) according to a conventional method, and by screening, a strain expressing about 100,000 IL-6 receptors per cell was established, and this strain was named "NJBC8". One clone of a hybridoma producing an antibody recognizing the cell contents of NJBC8 lysed by NP40 but not recognizing the cell contents of JURKAT lysed by NP40 was isolated and named "MT18". The monoclonal antibody produced by this hybridoma was named "MT18 antibody".

The above-mentioned hybridoma MT18 was deposited at the Fermentation Research Institute (FRI) Agency of Industrial Science and Technology 1-3 Yatabe-cho Higashi 1-chome Ibaraki Japan as FERM P-10840 on July 12, 1989, and transferred to an international deposition under the Budapest Treaty as FERM BP-2999 on July 10, 1990.

The cell line AM64 was deposited with the Fermention Research Institute (FRI) Agency of Industrial Science and Technology, located at 1-3 Yatabe-cho Higashi 1-chome Ibaraki Japan, on January 12, 1990, as FERM P-11194; and the cell line AM277 was deposited with the FRI on January 12, 1990, as FERM P-11195. Availability of deposited materials is not necessary for practice of the present invention, which may be performed using the teachings of the present disclosure in combination with publicly available materials and information and techniques well known in the arts of molecular biology, recombinant DNA, and chimeric gene expression.

Claims

1. Recombinant human gp130 protein which bonds to the IL-6 receptor in the presence of IL-6 but does not bond to the IL-6 receptor in the absence of IL-6.

2. Human gp130 protein having at least the following properties:

(1) the protein has an affinity with a complex of IL-6 (interleukin-6) and an IL-6 receptor (interleukin-6 receptor);

(2) the protein shows an apparent molecular weight of 130 kDa in SDS-polyacrylamide electrophoresis; and

(3) the protein participtes in the transmission of Il-6 signal.

3. Human gp130 protein as claimed in Claim 1 or Claim 2, characterized in that it has an amino acid sequence from Met at the 1st site to Gln at the 918th position in Figure 7.

4. DNA coding for human gp130 protein as claimed in Claim 1, 2 or 3.

5. DNA as claimed in Claim 4, characterized in that it codes for a human gp130 protein having an amino acid sequence of from Met at the 1st position to Gln at the 918th position in Figure 7.

6. DNA as claimed in Claim 5, characterized in that it has a nucleotide sequence from A at the 273rd position to G at the 3026th position in Figure 7.

7. A vector containing DNA as claimed in Claim 4, 5 or 6.

8. A vector as claimed in Claim 7 which is an expression vector capable of expressing the said DNA in a host cell.

9. The plasmid pPG130.

10. A host cell transformed by an expression vector as claimed in Claim 8.

11. A process for the production of human gp130 protein, which comprises culturing a host cell as claimed in Claim 10.

12. Human gp130 protein substantially free of another human protein, which is produced by using DNA as claimed in Claim 4, 5 or 6.

SEQ ID NO : 1
SEQUENCE TYPE : Nucleotide sequence with corresponding proteins
SEQUENCE LENGTH : 3102 base pairs

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GAATTCGGGG CCGGGGCGAG CAGCCAAAAG GCCCGCGGAG TCGCGCTGGG      50

CCGCCCCGGC GCAGCTGAAC CGGGGGCCGC GCCTGCCAGG CCGACGGGTC     100

TGGCCCAGCC TGGCGCCAAG GGGTTCGTGC GCTGTGGAGA CGCGGAGGGT     150

CGAGGCGGCG CGGCCTGAGT GAAACCCAAT GGAAAAAGCA TGACATTTAG     200

AAGTAGAAGA CTTAGCTTCA AATCCCTACT CCTTCACTTA CTAATTTTGT     250

GATTTGGAAA TATCCGCGCA AG ATG TTG ACG TTG CAG ACT TGG       293
                         Met Leu Thr Leu Gln Thr Trp
                                                5

GTA GTG CAA GCC TTG TTT ATT TTC CTC ACC ACT GAA TCT ACA    335
Val Val Gln Ala Leu Phe Ile Phe Leu Thr Thr Glu Ser Thr
         10                  15                  20

GGT GAA CTT CTA GAT CCA TGT GGT TAT ATC AGT CCT GAA TCT    377
Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro Glu Ser
            25                  30                  35

CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG    419
Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val
                40                  45

CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT    461
Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn
 50                  55                  60

TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG    503
Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu
     65                  70                  75

CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT    545
Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe
         80                  85                  90

ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT    587
Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile
            95                  100                 105

CTT ACA TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA    629
Leu Thr Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr
                110                 115

ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT    671
Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser
120                 125                 130
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TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG TGG GAT     713
Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu Trp Asp
    135                 140                 145

GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA     755
Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys
        150                 155                 160

TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA     797
Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys
            165                 170                 175

CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG     839
Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val
                180                 185

TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC     881
Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala
190                 195                 200

CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA     923
Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val
    205                 210                 215

TAT AAA GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC     965
Tyr Lys Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile
        220                 225                 230

AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC    1007
Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr
            235                 240                 245

AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT AAC ATT
Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr Asn Ile    1049
                250                 255

CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT    1091
Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro
260                 265                 270

CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA    1133
Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln
    275                 280                 285

GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT    1175
Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys
        290                 295                 300
```

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ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA   1217
Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu
            305                 310                 315

GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA   1259
Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala
                320                 325

CCA AGT TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC   1301
Pro Ser Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly
330                 335                 340


TAC AGA ACT GTA CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT   1343
Tyr Arg Thr Val Gln Leu Val Trp Lys Thr Leu Pro Pro Phe
    345                 350                 355

GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA GTG ACT CTC ACA   1385
Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val Thr Leu Thr
        360                 365                 370

AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT GCC ACA   1427
Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala Thr
            375                 380                 385

AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC   1469
Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr
                390                 395

CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT   1511
Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val
400                 405                 410

TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ATC CAC CCT GTA   1553
Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val
    415                 420                 425

ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG   1595
Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val
        430                 435                 440

GAA TGG ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT   1637
Glu Trp Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu
            445                 450                 455

GAG TGG TGT GTG TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC   1679
Glu Trp Cys Val Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp
                460                 465
```

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TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT TTA AGA   1721
Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr Leu Arg
470                 475                 480

GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT   1763
Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr
    485                 490                 495

CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG   1805
Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys
        500                 505                 510

GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT   1847
Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val
            515                 520                 525

CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG   1889
Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp
                530                 535

GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT   1931
Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn
540                 545                 550

TAT ACT ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT   1973
Tyr Thr Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala
    555                 560                 565

GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT   2015
Val Asn Val Asp Ser Ser His Thr Glu Tyr Thr Leu Ser Ser
        570                 575                 580

TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA GCA TAC   2057
Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala Ala Tyr
            585                 590                 595

ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT   2099
Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr
                600                 605

ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA GCC ATA GTC GTG   2141
Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val
610                 615                 620

CCT GTT TGC TTA GCA TTC CTA TTG ACA ACT CTT CTG GGA GTG   2183
Pro Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val
    625                 630                 635

CTG TTC TGC TTT AAT AAG CGA GAC CTA ATT AAA AAA CAC ATC   2225
Leu Phe Cys Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile
        640                 645                 650
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TGG CCT AAT GTT CCA GAT CCT TCA AAG AGT CAT ATT GCC CAG    2267
Trp Pro Asn Val Pro Asp Pro Ser Lys Ser His Ile Ala Gln
            655                 660                 665

TGG TCA CCT CAC ACT CCT CCA AGG CAC AAT TTT AAT TCA AAA    2309
Trp Ser Pro His Thr Pro Pro Arg His Asn Phe Asn Ser Lys
                670                 675

GAT CAA ATG TAT TCA GAT GGC AAT TTC ACT GAT GTA AGT GTT    2351
Asp Gln Met Tyr Ser Asp Gly Asn Phe Thr Asp Val Ser Val
680                 685                 690

GTG GAA ATA GAA GCA AAT GAC AAA AAG CCT TTT CCA GAA GAT    2393
Val Glu Ile Glu Ala Asn Asp Lys Lys Pro Phe Pro Glu Asp
    695                 700                 705

CTG AAA TCA TTG GAC CTG TTC AAA AAG GAA AAA ATT AAT ACT    2435
Leu Lys Ser Leu Asp Leu Phe Lys Lys Glu Lys Ile Asn Thr
        710                 715                 720

GAA GGA CAC AGC AGT GGT ATT GGG GGG TCT TCA TGC ATG TCA    2477
Glu Gly His Ser Ser Gly Ile Gly Gly Ser Ser Cys Met Ser
            725                 730                 735

TCT TCT AGG CCA AGC ATT TCT AGC AGT GAT GAA AAT GAA TCT    2519
Ser Ser Arg Pro Ser Ile Ser Ser Ser Asp Glu Asn Glu Ser
                740                 745

TCA CAA AAC ACT TCG AGC ACT GTC CAG TAT TCT ACC GTG GTA    2561
Ser Gln Asn Thr Ser Ser Thr Val Gln Tyr Ser Thr Val Val
750                 755                 760

CAC AGT GGC TAC AGA CAC CAA GTT CCG TCA GTC CAA GTC TTC    2603
His Ser Gly Tyr Arg His Gln Val Pro Ser Val Gln Val Phe
    765                 770                 775

TCA AGA TCC GAG TCT ACC CAG CCC TTG TTA GAT TCA GAG GAG    2645
Ser Arg Ser Glu Ser Thr Gln Pro Leu Leu Asp Ser Glu Glu
        780                 785                 790

CGG CCA GAA GAT CTA CAA TTA GTA GAT CAT GTA GAT GGC GGT    2687
Arg Pro Glu Asp Leu Gln Leu Val Asp His Val Asp Gly Gly
            795                 800                 805
```

```
GAT GGT ATT TTG CCC AGG CAA CAG TAC TTC AAA CAG AAC TGC   2729
Asp Gly Ile Leu Pro Arg Gln Gln Tyr Phe Lys Gln Asn Cys
                810                 815

AGT CAG CAT GAA TCC AGT CCA GAT ATT TCA CAT TTT GAA AGG   2771
Ser Gln His Glu Ser Ser Pro Asp Ile Ser His Phe Glu Arg
820                 825                 830

TCA AAG CAA GTT TCA TCA GTC AAT GAG GAA GAT TTT GTT AGA   2813
Ser Lys Gln Val Ser Ser Val Asn Glu Glu Asp Phe Val Arg
    835                 840                 845

CTT AAA CAG CAG ATT TCA GAT CAT ATT TCA CAA TCC TGT GGA   2855
Leu Lys Gln Gln Ile Ser Asp His Ile Ser Gln Ser Cys Gly
        850                 855                 860

TCT GGG CAA ATG AAA ATG TTT CAG GAA GTT TCT GCA GCA GAT   2897
Ser Gly Gln Met Lys Met Phe Gln Glu Val Ser Ala Ala Asp
            865                 870                 875

GCT TTT GGT CCA GGT ACT GAG GGA CAA GTA GAA AGA TTT GAA   2939
Ala Phe Gly Pro Gly Thr Glu Gly Gln Val Glu Arg Phe Glu
                880                 885

ACA GTT GGC ATG GAG GCT GCG ACT GAT GAA GGC ATG CCT AAA   2981
Thr Val Gly Met Glu Ala Ala Thr Asp Glu Gly Met Pro Lys
890                 895                 900

AGT TAC TTA CCA CAG ACT GTA CGG CAA GGC GGC TAC ATG CCT   3023
Ser Tyr Leu Pro Gln Thr Val Arg Gln Gly Gly Tyr Met Pro
    905                 910                 915

CAG TGAAGGACTA GTAGTTCCTG CTACAACTTC AGCAGTACCT          3066
Gln
918

ATAAAGTAAA GCTAAAATGA TTTTATCTGT GAATTC                  3102
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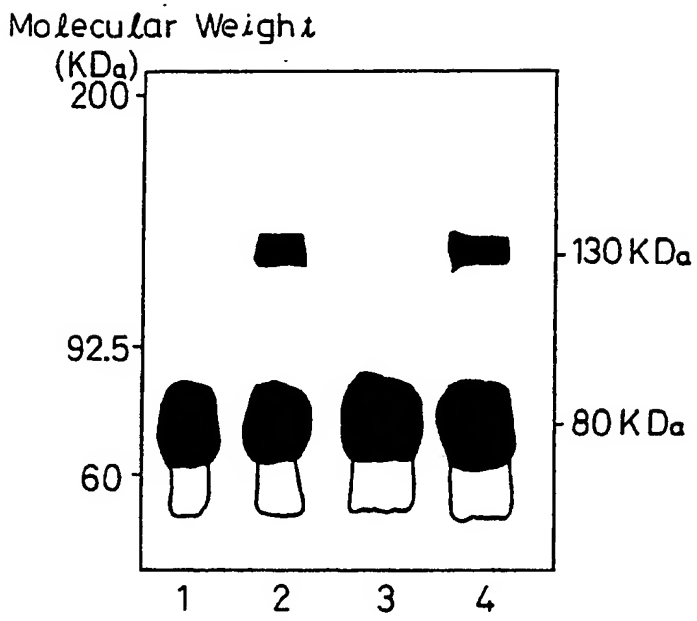
Fig. 1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(KDa)
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92.5
60
130 KDa
80 KDa
1
2
3
4

Fig. 2

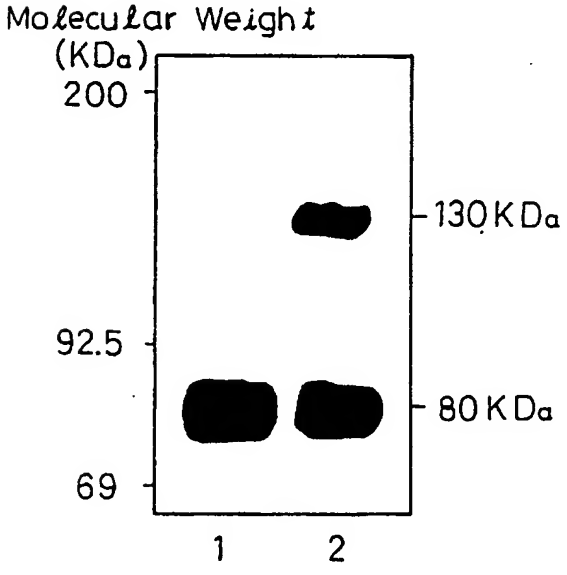
Molecular Weight
(KDa)
200
92.5
69
130 KDa
80 KDa
1
2

Fig. 3

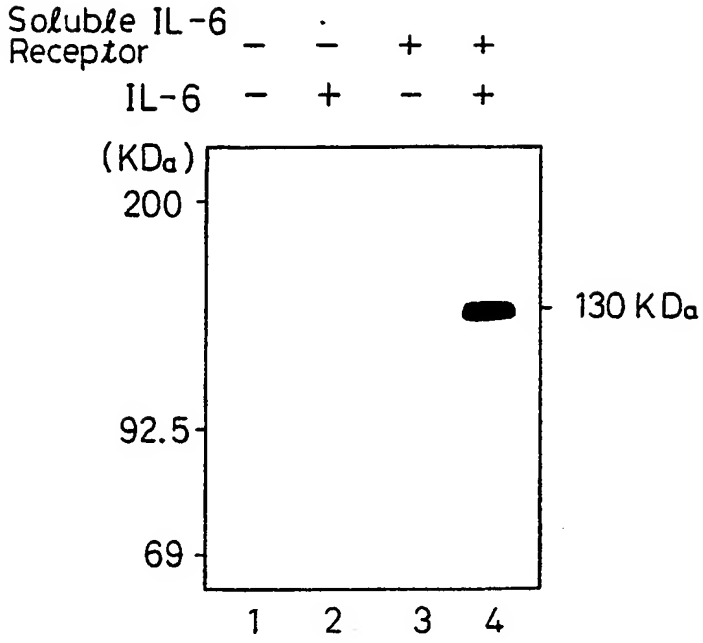
Soluble IL-6 Receptor
IL-6
(KDa)
200
92.5
69
130 KDa
1 2 3 4

Fig. 4

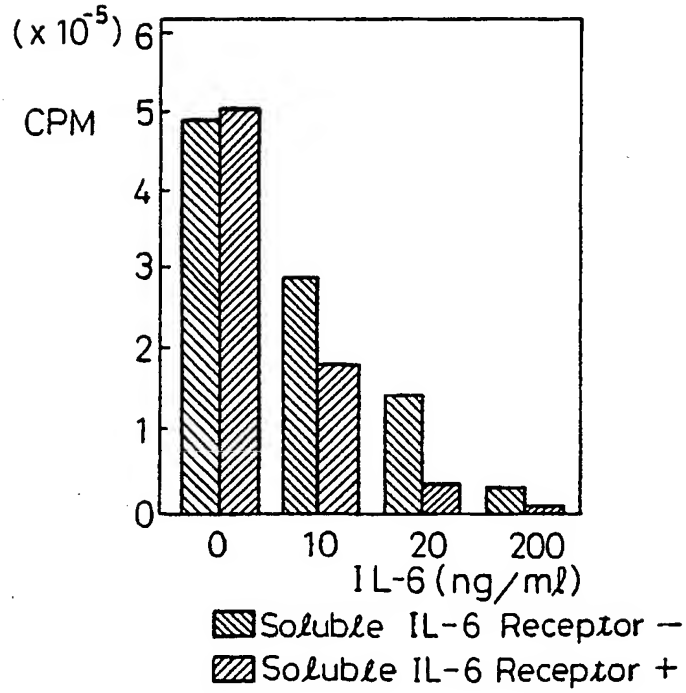
(x 10⁻⁵)
CPM
6
5
4
3
2
1
0
0 10 20 200
IL-6 (ng/ml)
Soluble IL-6 Receptor −
Soluble IL-6 Receptor +

Fig. 5

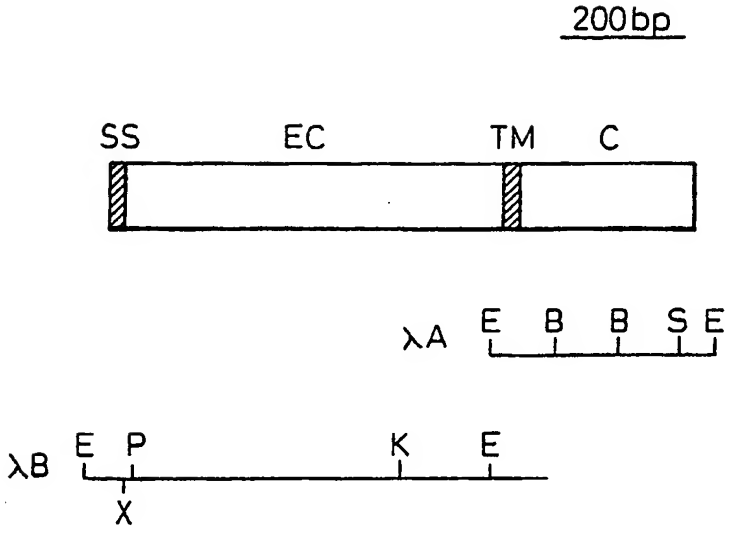
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Fig. 6

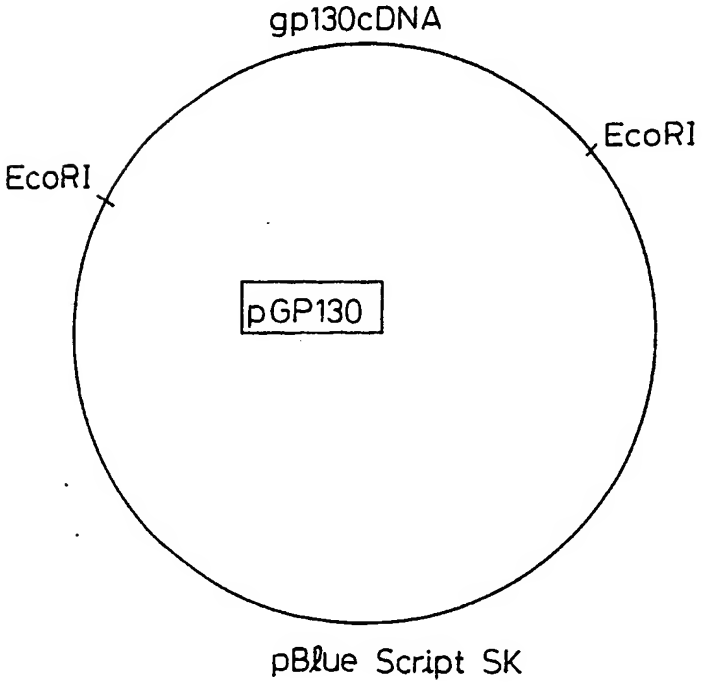
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Fig. 7-1

SEQUENCE ID NO 1

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CCGCCCCGGC GCAGCTGAAC CGGGGGCCGC GCCTGCCAGG CCGACGGGTC     100

TGGCCCAGCC TGGCGCCAAG GGGTTCGTGC GCTGTGGAGA CGCGGAGGGT     150

CGAGGCGGCG CGGCCTGAGT GAAACCCAAT GGAAAAAGCA TGACATTTAG     200

AAGTAGAAGA CTTAGCTTCA AATCCCTACT CCTTCACTTA CTAATTTTGT     250

                                                5
                          Met Leu Thr Leu Gln Thr Trp
GATTTGGAAA TATCCGCGCA AG ATG TTG ACG TTG CAG ACT TGG      293

          10                  15                  20
Val Val Gln Ala Leu Phe Ile Phe Leu Thr Thr Glu Ser Thr
GTA GTG CAA GCC TTG TTT ATT TTC CTC ACC ACT GAA TCT ACA    335

              25                  30                  35
Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro Glu Ser
GGT GAA CTT CTA GAT CCA TGT GGT TAT ATC AGT CCT GAA TCT    377

                  40                  45
Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val
CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG    419

 50                  55                  60
Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn
CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT    461

     65                  70                  75
Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu
TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG    503

          80                  85                  90
Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe
CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT    545

              95                 100                 105
Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile
ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT    587

                 110                 115
Leu Thr Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr
CTT ACA TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA    629

120                 125                 130
Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser
ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT    671
```

Fig. 7-2

```
    135                 140                 145
Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu Trp Asp
TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG TGG GAT    713

        150                 155                 160
Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys
GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA    755

            165                 170                 175
Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys
TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA    797

                180                 185
Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val
CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG    839

190                 195                 200
Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala
TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC    881

    205                 210                 215
Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val
CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA    923

        220                 225                 230
Tyr Lys Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile
TAT AAA GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC    965

            235                 240                 245
Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr
AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC   1007

                250                 255
Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr Asn Ile   1049
AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT AAC ATT

260                 265                 270
Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro
CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT   1091

    275                 280                 285
Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln
CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA   1133

        290                 295                 300
Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys
GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT   1175
```

Fig.7-3

```
                305                 310                 315
Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu
ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA   1217

                320                 325
Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala
GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA   1259

330                 335                 340
Pro Ser Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly
CCA AGT TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC   1301

    345                 350                 355
Tyr Arg Thr Val Gln Leu Val Trp Lys Thr Leu Pro Pro Phe
TAC AGA ACT GTA CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT   1343

        360                 365                 370
Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val Thr Leu Thr
GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA GTG ACT CTC ACA   1385

                375                 380                 385
Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala Thr
AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT GCC ACA   1427

                390                 395
Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr
AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC   1469

400                 405                 410
Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val
CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT   1511

    415                 420                 425
Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val
TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA   1553

        430                 435                 440
Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val
ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG   1595

                445                 450                 455
Glu Trp Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu
GAA TGG ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT   1637

                460                 465
Glu Trp Cys Val Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp
GAG TGG TGT GTG TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC   1679
```

Fig. 7-4

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470                 475                 480
Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr Leu Arg
TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT TTA AGA   1721

    485                 490                 495
Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr
GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT   1763

        500                 505                 510
Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys
CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG   1805

            515                 520                 525
Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val
GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT   1847

                530                 535
Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp
CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG   1889

540                 545                 550
Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn
GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT   1931

    555                 560                 565
Tyr Thr Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala
TAT ACT ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT   1973

        570                 575                 580
Val Asn Val Asp Ser Ser His Thr Glu Tyr Thr Leu Ser Ser
GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT   2015

            585                 590                 595
Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala Ala Tyr
TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA GCA TAC   2057

                600                 605
Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr
ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT   2099

610                 615                 620
Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val
ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA GCC ATA GTC GTG   2141

    625                 630                 635
Pro Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val
CCT GTT TGC TTA GCA TTC CTA TTG ACA ACT CTT CTG GGA GTG   2183

        640                 645                 650
Leu Phe Cys Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile
CTG TTC TGC TTT AAT AAG CGA GAC CTA ATT AAA AAA CAC ATC   2225
```

Fig.7-5

```
                655                 660                 665
Trp Pro Asn Val Pro Asp Pro Ser Lys Ser His Ile Ala Gln
TGG CCT AAT GTT CCA GAT CCT TCA AAG AGT CAT ATT GCC CAG   2267

                670                 675
Trp Ser Pro His Thr Pro Pro Arg His Asn Phe Asn Ser Lys
TGG TCA CCT CAC ACT CCT CCA AGG CAC AAT TTT AAT TCA AAA   2309

680                 685                 690
Asp Gln Met Tyr Ser Asp Gly Asn Phe Thr Asp Val Ser Val
GAT CAA ATG TAT TCA GAT GGC AAT TTC ACT GAT GTA AGT GTT   2351

    695                 700                 705
Val Glu Ile Glu Ala Asn Asp Lys Lys Pro Phe Pro Glu Asp
GTG GAA ATA GAA GCA AAT GAC AAA AAG CCT TTT CCA GAA GAT   2393

        710                 715                 720
Leu Lys Ser Leu Asp Leu Phe Lys Lys Glu Lys Ile Asn Thr
CTG AAA TCA TTG GAC CTG TTC AAA AAG GAA AAA ATT AAT ACT   2435

                725                 730                 735
Glu Gly His Ser Ser Gly Ile Gly Gly Ser Ser Cys Met Ser
GAA GGA CAC AGC AGT GGT ATT GGG GGG TCT TCA TGC ATG TCA   2477

                740                 745
Ser Ser Arg Pro Ser Ile Ser Ser Ser Asp Glu Asn Glu Ser
TCT TCT AGG CCA AGC ATT TCT AGC AGT GAT GAA AAT GAA TCT   2519

750                 755                 760
Ser Gln Asn Thr Ser Ser Thr Val Gln Tyr Ser Thr Val Val
TCA CAA AAC ACT TCG AGC ACT GTC CAG TAT TCT ACC GTG GTA   2561

    765                 770                 775
His Ser Gly Tyr Arg His Gln Val Pro Ser Val Gln Val Phe
CAC AGT GGC TAC AGA CAC CAA GTT CCG TCA GTC CAA GTC TTC   2603

        780                 785                 790
Ser Arg Ser Glu Ser Thr Gln Pro Leu Leu Asp Ser Glu Glu
TCA AGA TCC GAG TCT ACC CAG CCC TTG TTA GAT TCA GAG GAG   2645

                795                 800                 805
Arg Pro Glu Asp Leu Gln Leu Val Asp His Val Asp Gly Gly
CGG CCA GAA GAT CTA CAA TTA GTA GAT CAT GTA GAT GGC GGT   2687
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Fig. 7-6

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                  810                 815
Asp Gly Ile Leu Pro Arg Gln Gln Tyr Phe Lys Gln Asn Cys
GAT GGT ATT TTG CCC AGG CAA CAG TAC TTC AAA CAG AAC TGC   2729

820                 825                 830
Ser Gln His Glu Ser Ser Pro Asp Ile Ser His Phe Glu Arg
AGT CAG CAT GAA TCC AGT CCA GAT ATT TCA CAT TTT GAA AGG   2771

    835                 840                 845
Ser Lys Gln Val Ser Ser Val Asn Glu Glu Asp Phe Val Arg
TCA AAG CAA GTT TCA TCA GTC AAT GAG GAA GAT TTT GTT AGA   2813

        850                 855                 860
Leu Lys Gln Gln Ile Ser Asp His Ile Ser Gln Ser Cys Gly
CTT AAA CAG CAG ATT TCA GAT CAT ATT TCA CAA TCC TGT GGA   2855

            865                 870                 875
Ser Gly Gln Met Lys Met Phe Gln Glu Val Ser Ala Ala Asp
TCT GGG CAA ATG AAA ATG TTT CAG GAA GTT TCT GCA GCA GAT   2897

                  880                 885
Ala Phe Gly Pro Gly Thr Glu Gly Gln Val Glu Arg Phe Glu
GCT TTT GGT CCA GGT ACT GAG GGA CAA GTA GAA AGA TTT GAA   2939

890                 895                 900
Thr Val Gly Met Glu Ala Ala Thr Asp Glu Gly Met Pro Lys
ACA GTT GGC ATG GAG GCT GCG ACT GAT GAA GGC ATG CCT AAA   2981

    905                 910                 915
Ser Tyr Leu Pro Gln Thr Val Arg Gln Gly Gly Tyr Met Pro
AGT TAC TTA CCA CAG ACT GTA CGG CAA GGC GGC TAC ATG CCT   3023

918
Gln
CAG TGAAGGACTA GTAGTTCCTG CTACAACTTC AGCAGTACCT          3066

ATAAAGTAAA GCTAAAATGA TTTTATCTGT GAATTC                  3102
```
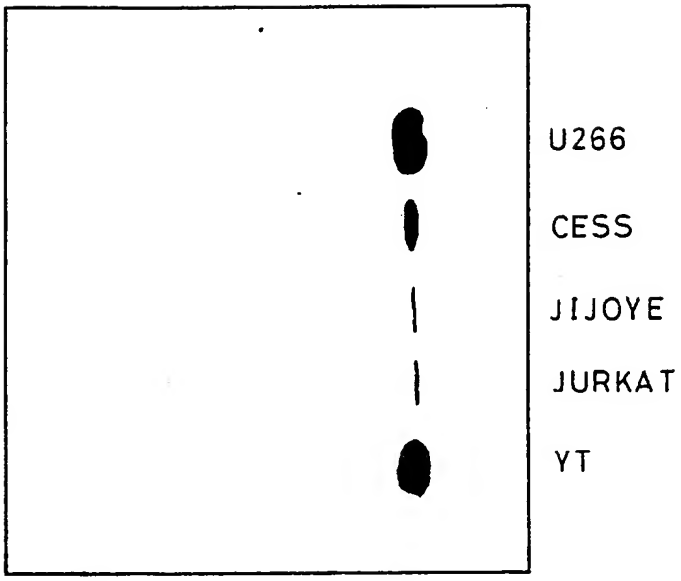

Fig. 8



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(71) Applicant: **Kishimoto, Tadamitsu**
5-31 Nakanocho 3-chome
Tondabayashi-shi Osaka(JP)

(72) Inventor: **Kishimoto, Tadamitsu**
5-31 Nakanocho 3-chome
Tondabayashi-shi Osaka(JP)

(74) Representative: **Kearney, Kevin David Nicholas et al**
KILBURN & STRODE 30 John Street
London, WC1N 2DD(GB)

(54) **Human GP130 protein.**

(57) Human gp130 protein having at least the following properties: (1) the protein has an affinity with a complex of IL-6 (interleukin-6) and an IL-6 receptor (interleukin-6 receptor); (2) the protein shows an apparent molecular weight of 130 kDa in SDS-polyacryla mide electrophoresis; and (3) the protein participates in the transmission of IL-6 signal; DNA coding for the protein, an expression plasmid containing the DNA; and a process for production of the protein using the expression plasmid.

EP 0 411 946 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 8530

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
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The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	19 November 91	HORNIG H.O.

CATEGORY OF CITED DOCUMENTS

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